Response to invited commentary : Vitamin D_3 supplementation for 8 weeks leads to improved haematological status following the consumption of an iron-fortified breakfast cereal: a double-blind randomised controlled trial in iron-deficient women. (1-2)

Salma F Ahmad Fuzi[1 2], Sohail Mushtaq[1]

(1) From the Faculty of Medicine, Dentistry and Life Sciences, University of Chester, Parkgate Road, Chester, UK, CH1 4BJ

(2) From the Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

(3) Address correspondence to Associate Professor Sohail Mushtaq, Faculty of Medicine, Dentistry and Life Sciences, University of Chester, Parkgate Road, Chester, UK, CH1 4BJ. Tel: +44 (0)124 4513367, Fax: +44 (0)124511310, Email address: s.mushtaq@chester.ac.uk

We would like to respond to the invited commentary by Blanco-Rojo[1] pertaining to our published findings on the improvement of iron-status indices following an eight-week vitamin D supplementation with iron-fortified cereals in UK iron-deficient women[2]. The key findings of our study supported the study hypothesis and suggest a role for vitamin D in iron regulation. In particular, our study demonstrated a significant effect of vitamin D3 supplementation in improving the participants' iron status, indicated by the increase in haemoglobin concentrations and haematocrit levels, despite the non-significant changes in hepcidin and ferritin concentrations. The commentary author correctly pointed out that low hepcidin concentration may have hindered the detection of a possible decrease. This is plausible, as the recruited participants for the study were iron deficient. Ganz *et al.*[3] who developed and validated the first ELISA specific for hepcidin, reported that serum hepcidin concentrations were undetectable in 18-19 iron-deficient patients. Multiple clinical factors that may be present simultaneously, have been shown to influence the concentration of circulating hepcidin[4]. A potential decrease may be insufficient to be observed due to the lack of sensitivity of the immunoassay used in the study which actually recognises all isoforms of hepcidin (-20, -22, -24, and -25). Hepcidin exists in various isoforms[5], and hepcidin-25 has been distinctively identified to play a role in iron regulation[6] with unclear underlying mechanisms[7; 8].

Focusing on the use of breakfast cereals in the study, Blanco-Rojo[1] inferred that the presence of phytic acid, calcium, and casein in the milk, with the lack of ascorbic acid, might inhibit iron absorption, which resulted in no statistically significant increase in ferritin concentrations and this cannot be ruled out due to the nature of the phytate content of breakfast cereals in particular. However, depending on the conditions and chemical forms of iron itself (haem or non-haem)[9], the overall percentage of iron absorption for an individual is reported to be classically low, and ranges from as low as 5% to as high as 35%[10]. Previous studies that used cereal-based meals also reported a wide range of iron absorption, of between 0.56% and 18.8%[11; 12; 13; 14; 15; 16]. The amount of phytates in ready-made cereal products varied from 0.05% to 3.29%[17]. A molar ratio of < 1:1 for phytates and iron in a meal was proposed to counteract the inhibition effect of phytates on the iron absorption; however, it was shown that phytates can still interfere with iron absorption at a ratio of as low as 0.2:1[14]. However, no means of measuring phytates was carried out in our study, which should be taken into account in future research in order to make a fair comparison in terms of the effect of phytates in the intervention food product on iron absorption in general. Cereals and cereal products represent the highest percentage of food (38%) that contributes to iron intake among UK women based on the latest National Dietary Nutrition Survey (NDNS)[18], where high-fibre breakfast cereals represented 7%. This explains why it was selected as means of providing dietary

iron to the participants in our study. The use of semi-skimmed milk in the study provides approximately 250 mg of calcium with no clear underlying mechanism on how calcium affects iron uptake and bioavailability[19]. A dose-dependent effect of 40 to 600 mg of calcium in foods on iron absorption was observed in one of the earliest studies by Hallberg *et al.*[20]. In a recent study, Candia *et al.*[21] investigated the effects of various calcium salts on non-haem iron bioavailability in fasted women of childbearing age and found 800 mg of supplemental calcium citrate significantly decreased non-haem iron bioavailability. Additionally, several studies have reported conflicting findings, due to factors such as; variations in the forms of calcium; differing administration routes, including within foods in a single meal; complex meals; or as supplements; in addition to varied doses[20; 22; 23; 24; 25].

Blanco-Rojo[1] suggested that the food fortification vehicles used in previous studies may have contributed to low iron absorption in the first place, but the limited number of recent iron fortification studies carried out in various settings have reported discrepancies in the improvement of iron status biomarkers **(Table 1)**. There was no significant increase in the daily iron intake in our study, however, the intake was 1.7-fold higher at baseline, relative to typical iron intake of adult women reported in the NDNS, which accounted to approximately 116% of the UK RNI. We agree that future RCTs should consider the importance of addressing the use of food products that take into account the bioavailability of both iron and vitamin D. Findings from such studies have the potential to provide an alternative or adjunct route to manage iron deficiency, as opposed to the therapeutic oral iron therapy that can lead to poor adherence due to the adverse gastrointestinal events.

Conflicts of interest

There were no conflicts of interest and this research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Table 1 Recent iron fortification studies using cereal and cereal-based products

Study	Population /Mean age (SD) or range	Intervention/Duration	Main findings/Comments
[1]	ID women (n=50) 27.4 (9.4) yrs	Iron-fortified cereal/ 8 weeks	Mean (SD) Hb concentration was significantly higher at post-intervention in the vitamin D group (13.84 (0.98) g/dl) compared to placebo group (12.83 (1.25) g/dl) ($p<0.05$).
[2]	Children (n=112) 6-12 yrs	Iron-fortified biscuits (high or low iron) 4 months	Mean (SD) Hb concentration was significantly higher at post-intervention in the high iron group (10.52 (0.81) g/dl) compared to low iron group (10.03 (0.73) g/dl) ($p<0.01$). The study showed Hb concentration can be increased in 4 months. The present study showed that Hb concentration is increased at a shorter duration of 8 weeks. The high iron group received 30 mg of iron/serving of biscuits, whilst iron-fortified cereal used in the present study consisted of 9 mg iron/serving.
[3]	Children (n=47) 3-6 yrs	A: Fortified biscuit (FS) B: Fortified biscuit (HIC) C: Placebo biscuit 10 weeks	Mean (SD) Hb concentration was significantly higher at post-intervention in A (14.9 (0.2) g/dl) and B (14.7 (0.3) g/dl) compared to placebo group (15.3 (0.3) g/dl) ($p<0.05$). Participants in group C were non-anaemic for comparison with anaemic participants in group A and B, hence the higher Hb concentration at post-intervention. None of the other iron status biomarkers measured in the study (RBC, MCV, MCH, MCHC, and SF) were affected by the intervention, comparable to the present study.
[4]	Adolescents (n=71) 6-19 yrs	Iron-fortified or unfortified breakfast cereals 12 weeks	Mean (SD) SF concentration was significantly higher at post-intervention in the intervention group (22.1 (16.7) µg/l) compared to unfortified group (18.4 (11.6) µg/l) ($p<0.001$). The study found no effect of intervention on the other iron biomarkers including Hb, Hct and MCV, contrary to the present study. The participants in the study were not ID as opposed to the present study, but were recruited based on riboflavin status (EGRAC > 1.4 and Hb < 13.7 g/dl). The significant observation reported in the study may be due to a longer study duration, as opposed to 8 weeks in the present study.

[1]Fuzi and Mushtaq[(2)] (UK); [2] Bal *et al.* [(26)] (India); [3] Quintero-Gutiérrez *et al* [(27)] (Mexico); [4] Powers *et al.*[(28)] (UK)
Abbreviations: Hb: haemoglobin; SF: serum ferritin; RBC: red blood cell; MCV: mean corpuscular volume: MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; FS: ferrous sulphate; HIC: haem iron concentrate; EGRAC: erythrocyte glutathione reductase activation coefficient

List of references:

1. Blanco-Rojo R (2019) Invited commentary in response to: Vitamin D 3 supplementation for 8 weeks leads to improved haematological status following the consumption of an iron-fortified breakfast cereal: a double-blind randomised controlled trial in iron-deficient women. *British Journal of Nutrition* **122**, 603-604.

2. Fuzi SFA, Mushtaq S (2019) Vitamin D 3 supplementation for 8 weeks leads to improved haematological status following the consumption of an iron-fortified breakfast cereal: a double-blind randomised controlled trial in iron-deficient women. *British Journal of Nutrition* **121**, 1146-1157.

3. Ganz T, Olbina G, Girelli D *et al.* (2008) Immunoassay for human serum hepcidin. *Blood* **112**, 4292-4297.

4. Girelli D, Nemeth E, Swinkels DW (2016) Hepcidin in the diagnosis of iron disorders. *Blood, The Journal of the American Society of Hematology* **127**, 2809-2813.

5. Hunter HN, Fulton DB, Ganz T *et al.* (2002) The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochromatosis. *Journal of Biological Chemistry* **277**, 37597-37603.

6. Lehtihet M, Bonde Y, Beckman L *et al.* (2016) Circulating hepcidin-25 is reduced by endogenous estrogen in humans. *PLoS One* **11**.

7. Qiao B, Sugianto P, Fung E *et al.* (2012) Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. *Cell metabolism* **15**, 918-924.

8. Ross SL, Tran L, Winters A *et al.* (2012) Molecular mechanism of hepcidin-mediated ferroportin internalization requires ferroportin lysines, not tyrosines or JAK-STAT. *Cell metabolism* **15**, 905-917.

9. McDowell LR (2003) *Minerals in animal and human nutrition*: Elsevier Science BV.

10. Abbaspour N, Hurrell R, Kelishadi R (2014) Review on iron and its importance for human health. *Journal of Research in Medical Sciences* **19**, 164-174.

11. Layrisse M, García-Casal MN, Solano L *et al.* (2000) Iron bioavailability in humans from breakfasts enriched with iron bis-glycine chelate, phytates and polyphenols. *The Journal of nutrition* **130**, 2195-2199.

12. Davidsson L, Walczyk T, Zavaleta N *et al.* (2001) Improving iron absorption from a Peruvian school breakfast meal by adding ascorbic acid or Na2EDTA. *The American Journal of Clinical Nutrition* **73**, 283.

13. Mendoza C, Viteri FE, Lönnerdal B *et al.* (2001) Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. *The American journal of clinical nutrition* **73**, 80-85.

14. Hurrell R, Reddy M, Burri J *et al.* (2002) Phytate degradation determines the effect of industrial processing and home cooking on iron absorption from cereal-based foods. *British Journal of Nutrition* **88**, 117-123.

15. Fidler MC, Davidsson L, Zeder C *et al.* (2004) Erythorbic acid is a potent enhancer of nonheme iron absorption. *The American journal of clinical nutrition* **79**, 99-102.
16. Ahmad Fuzi SF, Koller D, Bruggraber S *et al.* (2017) A 1-h time interval between a meal containing iron and consumption of tea attenuates the inhibitory effects on iron absorption: a controlled trial in a cohort of healthy UK women using a stable iron isotope. *The American journal of clinical nutrition* **106**, 1413-1421.
17. Reddy N (2002) Chapter 3: Occurrence, distribution, content, and dietary intake of phytate. In *Food phytates*, pp. 25-51 [N Reddy and S Shridhar, editors]. Florida, USA: CRC Press LLC.
18. Bates B, Lennox A, Prentice A *et al.* (2014) The National Diet and Nutrition Survey. Results from Years 1,2,3 and 4 (combined) of the Rolling Programme (2008/2009-2011/2012). London, United Kingdom: TSO.
19. Lynch SR (2000) The effect of calcium on iron absorption. *Nutrition research reviews* **13**, 141-158.
20. Hallberg L, Rossander-Hulthèn L, Brune M *et al.* (1993) Inhibition of haem-iron absorption in man by calcium. *British Journal of Nutrition* **69**, 533-540.
21. Candia V, Ríos-Castillo I, Carrera-Gil F *et al.* (2018) Effect of various calcium salts on non-heme iron bioavailability in fasted women of childbearing age. *Journal of Trace Elements in Medicine and Biology* **49**, 8-12.
22. Monsen ER, Hallberg L, Layrisse M *et al.* (1978) Estimation of available dietary iron. *The American Journal of Clinical Nutrition* **31**, 134-141.
23. Gleerup A, Rossanderhulthen L, Gramatkovski E *et al.* (1995) Iron absorption from the whole diet - comparison of the effecr of 2 different distributions of daily calcium intake. *The American Journal of Clinical Nutrition* **61**, 97-104.
24. Benkhedda K, L'Abbé MR, Cockell KA (2010) Effect of calcium on iron absorption in women with marginal iron status. *The British Journal of Nutrition* **103**, 742-748.
25. Grinder-Pedersen L, Bukhave K, Jensen M *et al.* (2004) Calcium from milk or calcium-fortified foods does not inhibit nonheme-iron absorption from a whole diet consumed over a 4-d period. *The American Journal of Clinical Nutrition* **80**, 404-409.
26. Bal D, Nagesh K, Surendra H *et al.* (2015) Effect of supplementation with iron fortified biscuits on the haemoglobin status of children in rural areas of Shimoga, Karnataka. *The Indian Journal of Pediatrics* **82**, 253-259.
27. Quintero-Gutiérrez AG, González-Rosendo G, Pozo JP *et al.* (2016) Haem iron concentrate and iron sulfate added to chocolate biscuits: effects on haematological indices of Mexican school children. *J Am Coll Nutr* **35**, 544-551.

28. Powers HJ, Stephens M, Russell J *et al.* (2016) Fortified breakfast cereal consumed daily for 12 wk leads to a significant improvement in micronutrient intake and micronutrient status in adolescent girls: a randomised controlled trial. *Nutrition Journal* **15**, 69.